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In Silico Analysis of Gene Expression Location and Single Nucleotide Polymorphisms (SNPs) of The Glucagon Like Peptide 1 Receptor (GLP-1R)

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ABSTRACT

Association studies of SNPs have become very important in determining how genetic variants are linked to complex diseases, quantitative traits, and physiological responses. Genetic polymorphisms in the GLP-1R gene have potentially decreased protein stability and are associated with many diseases, especially diabetes and obesity. This study aimed to screen the expression and investigate the genetic polymorphism of the GLP-1R by using several beneficial bioinformatics tools. We observed database Ensembl, GTEx portal, and KEGG to identify the structure, expression, and molecular pathway of GLP-1R. *In silico* computational methods (SIFT, PolyPhen v2, PROVEAN, and PhD-SNP) were used to identify nsSNPs of the GLP-1R that potentially influence protein structure and function. I-Mutant was used to investigate possibly damaging nsSNPs, while GeneMANIA was used to investigate GLP-1R gene-gene interactions. GLP-1R is localized on chromosome 6p21, contains 13 exons, and has the regulation variant (CTCF, promotor, enhancer, and promotor flank region). GLP-1R is highly expressed in the pancreas to stimulate glucose-dependent insulin secretion and suppress glucagon secretion. Seven nsSNPs of the GLP-1R gene were found to be potentially deleterious: rs10305421, rs201672448, rs10305492, rs2295006, rs6923761, rs1042044, rs140642887, and rs10305510. I-Mutant server showed that nsSNPs rs140642887 was unstable, decreased GLP-1R protein stability, and impaired other genes' interaction and function (SP1, SP3, GNAS, and GCG). This study is the first in silico analysis of the polymorphic GLP-1R gene, and will serve as a great resource for developing precision medications to treat diseases associated with these polymorphisms.

1. Introduction

The majority of genetic diversity in the human population is caused by single nucleotide polymorphisms (SNPs), defined as single-base variations in a DNA sequence. Genetic polymorphisms in the human genome may be found in both the coding (gene) and noncoding portions of the genome, and they are found at varying densities in each part of the genome (Lee *et al.* 2005). Single-nucleotide variants can alter phenotypic functions and potentially lead to disease development. SNPs in regulatory areas have been shown to affect gene expression or transcription factor binding (Kimura *et al.* 2012). Polymorphisms in coding DNA, particularly those that result in a change in the amino acid sequence of the protein product (nonsynonymous, nsSNP), are significant since they account for roughly half of all known genetic variants associated with hereditary illness in humans (Hampe *et al.* 2007).

The glucagon-like peptide-1 receptor (GLP-1R) belongs to the class B family of G protein-coupled peptide hormone receptors (GPCRs). Human GLP-1R is a 463-amino acid. These receptors' most important structural and functional feature is their large extracellular N-terminal domain (ECD). The ECD is a globular structure that forms trilayer α-β-βα folds that are held together by three pairs of cysteine disulphide bonds. Class B receptor activation

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necessitates the presence of these domains because they identify the ligand during the first binding process (Watkins *et al.* 2012). GLP-1's C terminus binds to the ECD structure in a peptide-binding groove accessible to numerous interactions. The GLP-1R is a major physiological regulator of insulin production, and it has the potential to be a significant therapeutic target in the treatment of type 2 diabetes.

GLP1-1R variants have been shown to alter insulin production in humans through their response to exogenous GLP-1 and to influence food intake in mice (Kumar *et al.* 2007). The GLP-1R SNPs rs3765467 and rs10305492 play an important role in regulating β cell insulin secretory capacity and mass (Weizheng *et al.* 2020). Some variations in the GLP-1R gene were associated with a lower risk of developing coronary artery disease (CAD) in type 2 diabetic patients from a Han Chinese population (Xiaouma *et al.* 2018). A previous study linked numerous genetic variations in a gastrointestinal hormone receptor to obesity phenotypes, appetite regulation, and metabolic disorders (Chiurazzi *et al.* 2020). GLP-1R gene rs1042044 is a promising candidate for future psychiatric studies of genetic markers that contribute to stress sensitivity, including associations between a novel GLP-1R genetic variant and hypothalamus-pituitary-adrenal (HPA) axis regulation (Sheikha *et al.* 2010).

Few studies have determined how these polymorphisms may contribute to disease development. In addition, the GLP-1R gene contains many unstudied SNPs that may be important in the aetiology of several diseases. Therefore, it is necessary to discover SNPs within the GLP-1R gene and assess the functional importance of these polymorphisms. This is the first study to perform a comprehensive *in silico* analysis of nsSNPs of the GLP-1R gene. This study may be relevant in the future for designing precision medicines to treat disorders caused by these genomic variants. In this study, we identified the expression of GLP-1R at various locations, collected missense nsSNPs of the GLP-1R gene, and screened the nsSNPs using a variety of bioinformatics software tools to determine the damaging nature of the nsSNPs of the GLP-1R gene.

2. Materials and Methods

The National Center for Biological Information (NCBI) Single Nucleotide Polymorphism (SNP) database (https://www.ncbi.nlm.nih.gov/SNP/) was used to access the SNPs of the GLP-1R gene data on humans (accessed January 17, 2022). Only missense nsSNPs were chosen from the NCBI SNP database as they can modify the amino acid sequence encoded by the protein and potentially disturb the structural arrangement and function of the proteins. Figure 1 illustrates the research methodology flow chart used in this study.

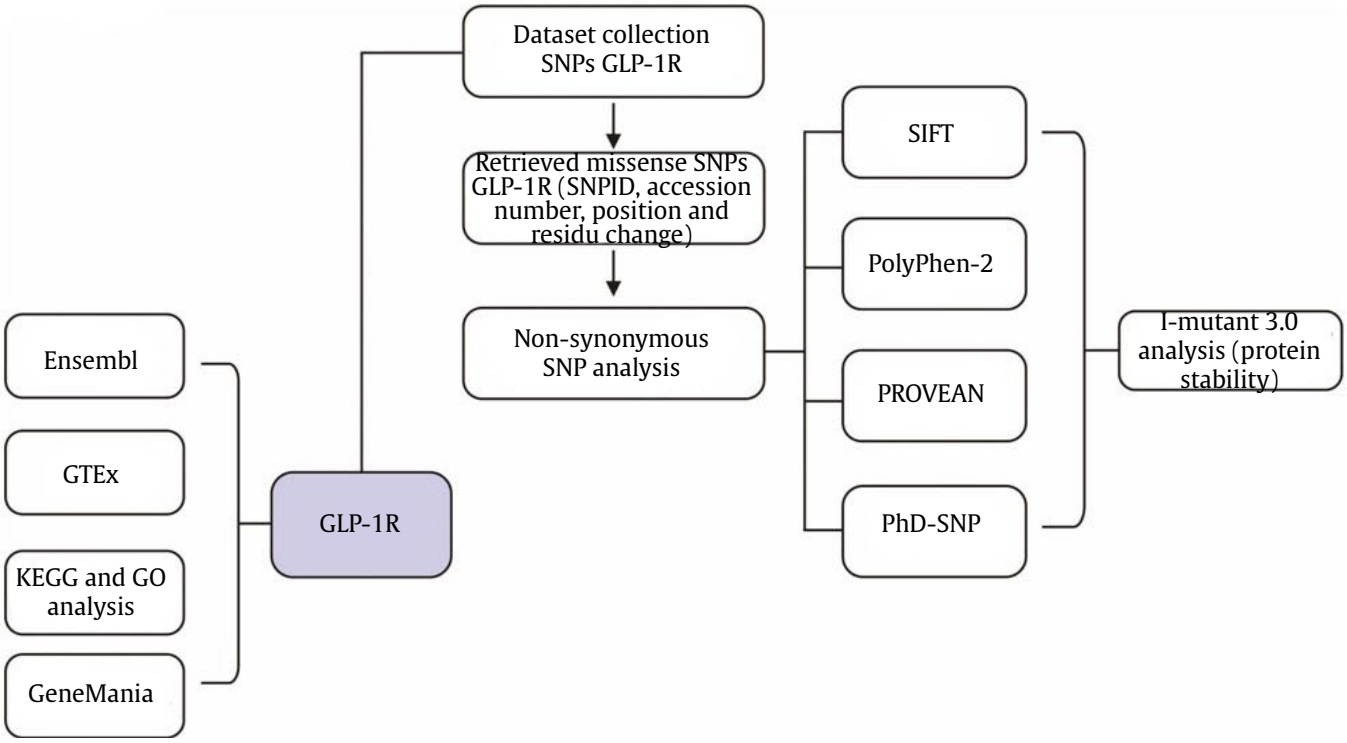

Figure 1. Flow chart of research methodology

2.1. Structure and Variations of the GLP-1R

The Ensemble genome browser (https://asia.ensembl.org/index.html) was used to examine the genomic structure and variations of the GLP-1R. The browser provides comprehensive information about comparative genomics, evolution, gene sequence and splice variant analysis, sequence variation analysis, transcriptional regulation, regulatory function, protein domains, and disorders for the majority of vertebrate species (http://www.ensembl.org/Homo_sapiens/Location/View?db=core;g=ENSG00000112164;r=6:39048781-39091303;t=ENST00000373256).

2.2. The Influence of Genetic Diversity on the Levels of Tissue-specific Gene and Exon Expression Using GTEx

Genotype-Tissue Expression (GTEx) information is available at https://gtexportal.org/home. It is comprehensive *in silico* tool that can predict inherited disease susceptibility by analyzing tissue-specific gene expression levels in response to genetic variation. The GTEx portal database was used to study tissue-specific gene expression and regulation of the GLP-1R gene in order to find the expression of GLP-1R genes. On January 17, 2022, the GTEx portal database was retrieved (https://gtexportal.org/home/gene/GLP1R).

2.3. KEGG and GO Analysis of GLP-1R-related Molecular Pathways

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a public online server that identifies biological pathways, illnesses, medications, and chemical compounds. GO (gene ontology) (http://geneontology.org/) is a comprehensive computational tool that provides useful information for different genes across all species, such as biological processes, molecular activities, cellular components, and molecular pathways. On January 17, 2022, the KEGG and GO analyses of GLP-1R were retrieved.

2.4. Characterization of SNPs

The NCBI website browser and the dbSNP database were utilized as databases for SNPs GLP-1R characterization. Information about non-synonymous SNPs (SNP ID, protein accession number, position, and residue change) was retrieved from the NCBI dbSNP database. All 356 nsSNPs were filtered for investigation. The dbSNP database database was retrieved on February 23, 2022 (https://www.ncbi.nlm.nih.gov/snp/?term=GLP1R).

2.5. Non-synonymous SNP Analysis

The following *in silico* algorithms and techniques were used to estimate the functional impact of nsSNPs:

a. Investigation of functional consequences of SNPs by sorting intolerant from tolerant (SIFT). Based on sequence alignment and homology, SIFT predicts whether a substitution of an amino acid will impair protein function. SIFT predicts the probability of observing a new amino acid at that specific position, with a score less than or equal to 0.05 being deleterious or damaging and a score greater than or equal to 0.05 being predicted to be tolerated (https://sift.bii.a-star.edu.sg/www/SIFT_help.html#SIFT).
b. Analysis of functional effects of SNPs by polymorphism phenotyping v2 (PolyPhen-2). PolyPhen-2 is a tool that uses simple physical and comparative considerations to predict the impact of an amino acid substitution on the structure and function of a human protein. Scores of 0.0 to 0.15, 0.15 to 0.85, and 0.85 to 1.0 are classified as benign, possibly damaging, and damaging, respectively (http://genetics.bwh.harvard.edu/pph2/).
c. Analysis of functional effects of SNPs by protein variation effect analyzer (PROVEAN). PROVEAN is a software tool that predicts whether an amino acid substitution or indel will affect a protein's biological function. A score of <-2.5 indicates a "deleterious" variant, while a score greater than >-2.5 indicates a "neutral" variant (http://provean.jcvi.org/index.php).
d. Prediction of functional impacts of NSPs by Predictor of human deleterious single nucleotide polymorphism (PhD-SNP).

Phd-SNP predicts disorder-causing SNPs by affecting protein function and structure. This service groups the effects of SNPs on disease, and scores range from 0 to 9. Protein sequence, location, and new residue are all essential inputs (https://snps.biofold.org/phd-snp/phd-snp.html). At least four in silico methods predicted harmful SNPs, designated high-risk nsSNPs, and subjected them to further investigation.

2.6. Prediction of nsSNPs Effects on Protein Stability by I-Mutant 3.0

The I-Mutant 3.0 tool was used to assess the effect of nsSNPs on protein stability. The tool uses data from ProTherm, the complete resource of experimental data on protein mutations. It forecasts the reliability index (RI) of the findings on a scale of 0–10, with ten being the most reliable. Delta Delta G (DDG) is the change in Gibbs free energy, and the difference in free energies of folded wild-type and mutant structures can predict the change in folding free energy. I-Mutant 3.0 outputs a DDG value that is classified as one of three predictions: mostly unstable (DDG 0.5 kcal/mol), largely stable (DDG > 0.5 kcal/mol), or neutral (0.5 DDG 0.5 kcal/mol). The GLP-1R protein sequence was submitted to predict the effects of the most damaging nsSNPs on the protein (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi).

2.7. Gene-gene Interaction

Gene MANIA (http://www.genemania.org) predicts gene function and generates information such as gene co-expression, co-localization, common protein domains, and pathways involved in gene function prediction. Gene MANIA predicted the gene-gene interaction network of the GLP-1R gene.

3. Results

The Ensemble has demonstrated that GLP-1R is localized on chromosome 6p21 with a position of 2139,016,574-39,055,519 forward strand (39.15 kb) and contains 13 exons (Figure 2 and 3). Figure 4A showed GLP-1R expression in several organs, scoring with log10 (Transcripts Per Million (TPM) +1). GLP-1R was highly expressed in the pancreas and had the lowest expression in the esophagus-gastroesophageal junction. GTEx has shown that the median read count

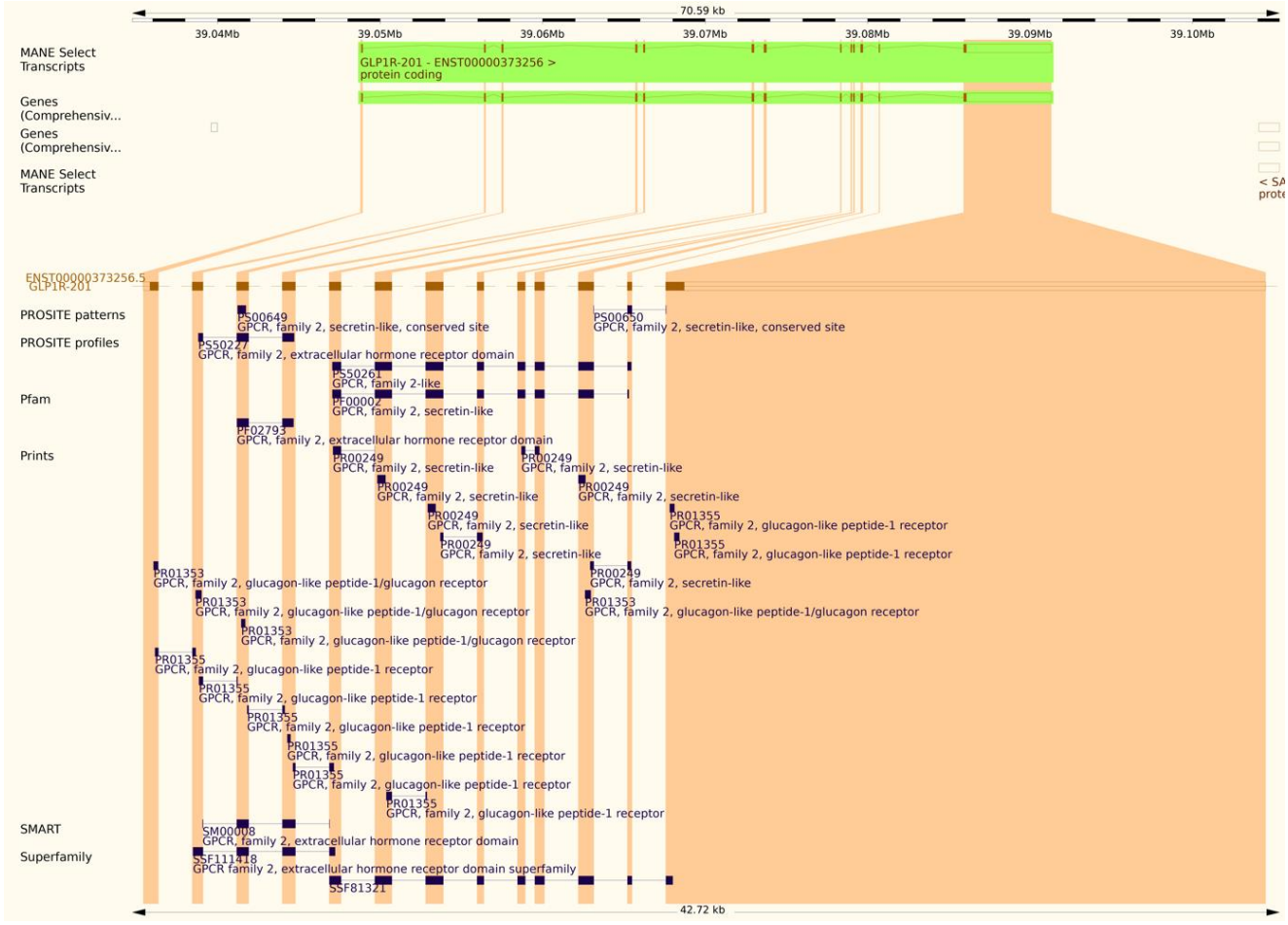

Figure 2. Splice variants of GLP-1R on choromosom 6p21

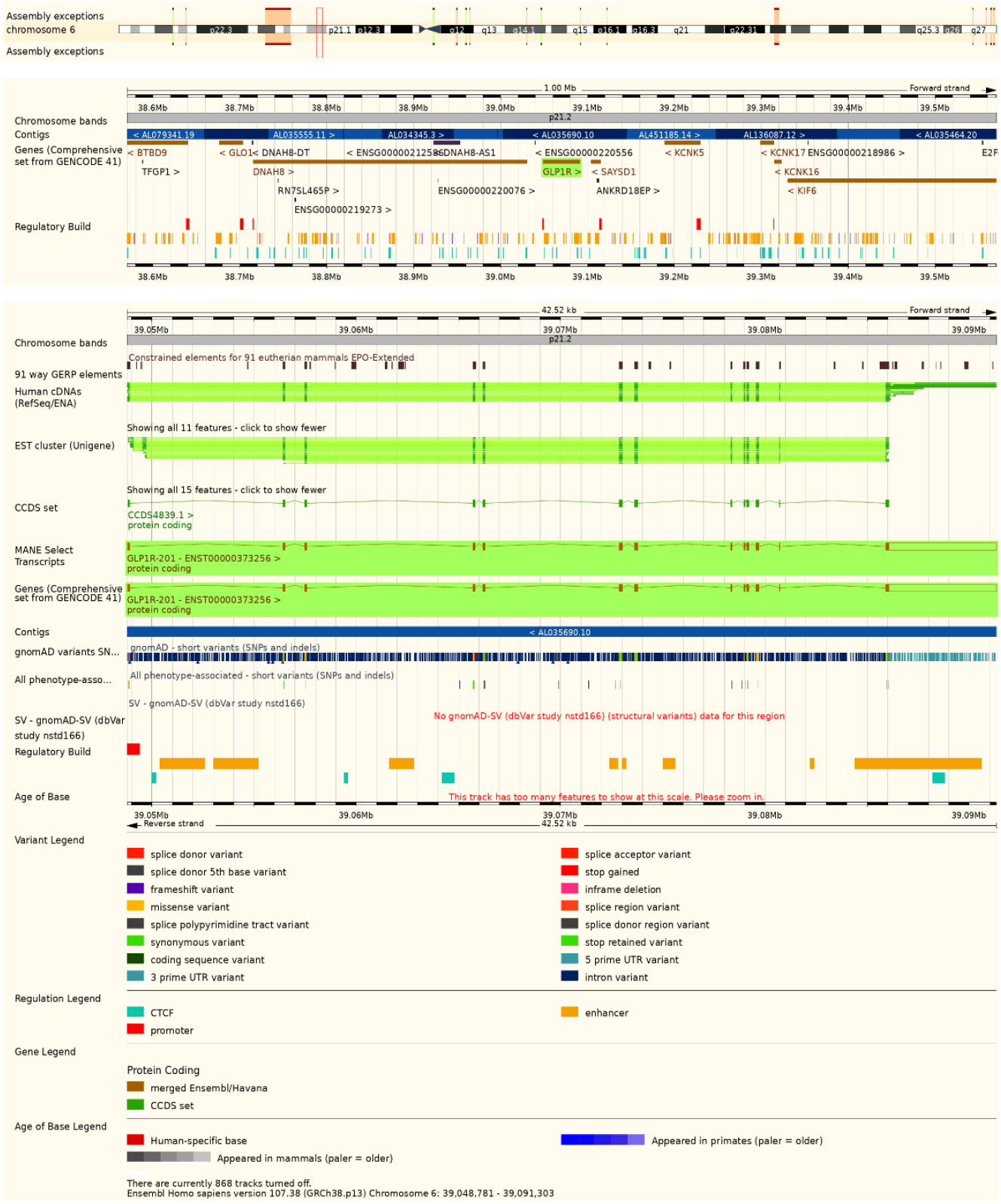

Figure 3. Ensembl gene map of GLP-1R and its variants in chromosome 6 (6p21)

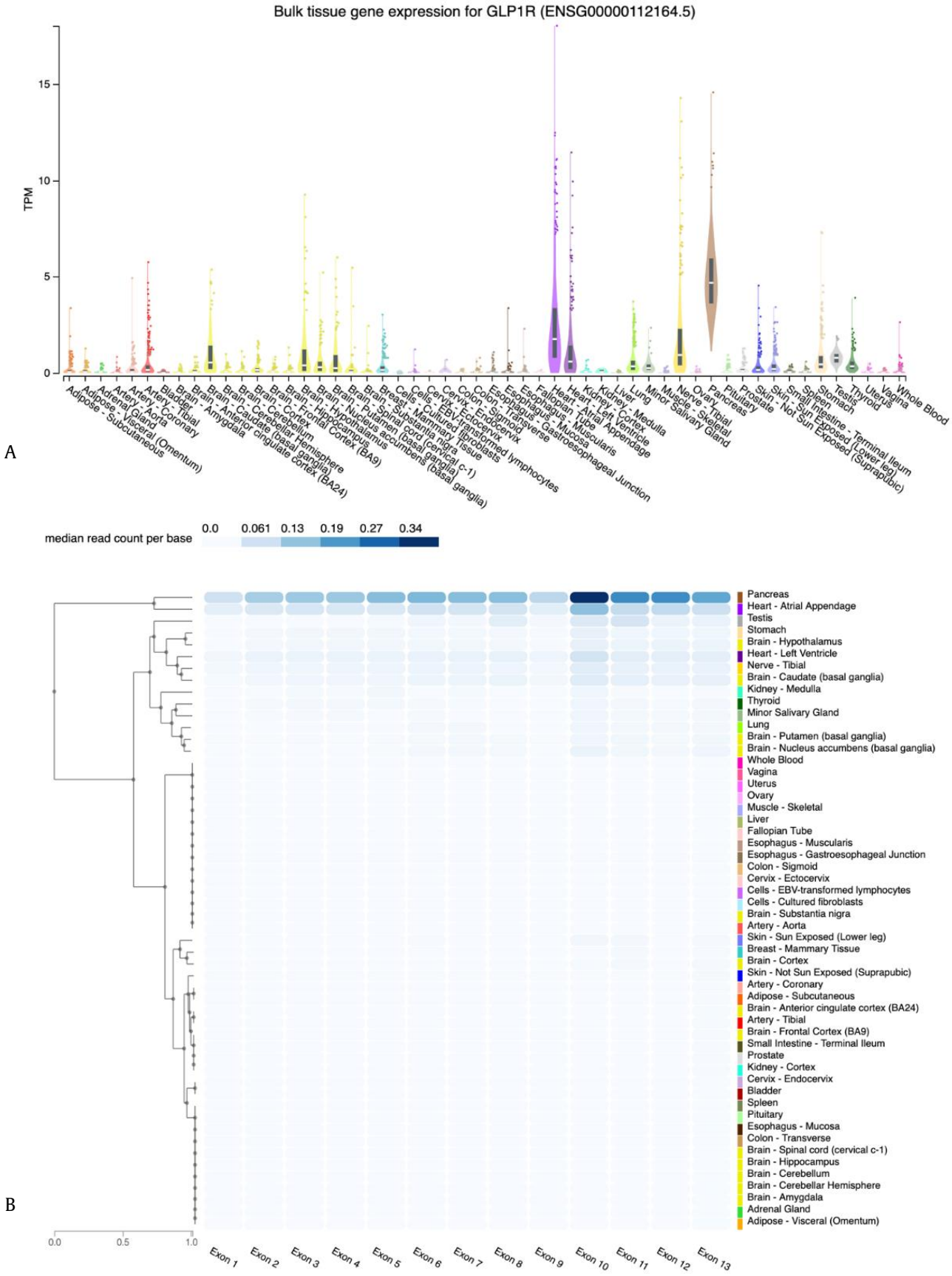

Figure 4. Location of GLP-1R in GTEx. (A) Comparison of GLP-1R expression levels in different tissues. (B) Exon expression of GLP-1R in several tissues with median read count per base score

per base for exons 11, 12, and 10 in the pancreas is significantly greater than other tissues (Figure 4B). Molecular functions and signalling pathways of GLP-1R were identified using the KEGG database (Figure 5). Furthermore, KEGG pathways revealed that the GLP-1R plays a role in the cAMP signalling pathway, neuroactive ligand-receptor interaction, and insulin secretion.

The National Center for Biotechnology Information (NCBI) search database identified all SNPs in the GLP-1R and their features, including variation type intron, missense, synonymous, inframe deletion, noncoding transcript variant minor allele frequency (MAF), and SNPs frequency in different groups. Obtained results from dbSNP showed that 16.399 SNPs were located within GLP-1R, including intronic (13.625), missense (356), synonymous (222), noncoding transcript variant (3820), and inframe deletion (4). We selected only nsSNPs for our investigation and limited them to SNPs with MAF > 0.001: therefore, 27 nsSNPs were obtained. We performed checks on the combined nsSNPs data. There were rs 229506 (1), rs 1042044 (5), rs 2235868 (2), rs 2765467 (1), rs 6918287 (2), rs 692376 (3), and rs 10305492 (1), rs 6918287 (2), rs 692376 (3), and rs 10305492 (1), respectively. Finally, we discovered 13 nsSNPs data for GLP-1R (Table 1 and 2). We used the I-Mutant bioinformatics tool to investigate whether nsSNPs rs140642887 increases or decreases GLP-1R protein stability (Table 3).

The interaction network of the GLP-1R gene as predicted by GeneMANIA is shown in Figure 6. The program assigned that the members of the association network be connected via these networks: physical interactions 77.64%, co-expression 8.01%, predicted 5.37%, co-localization 3.63%, genetic interactions 2.87%, pathway 1.88%, and shared protein domains 0.60%. The network weights add up to 100% and indicate the importance of each data source in predicting query list membership. The genes were ranked using these scores.

4. Discussion

The glucagon-like peptide one receptor (GLP-1R) is a G-protein-coupled receptor (GPCR) that binds with glucagon-like peptide-1 (GLP-1) as a protein-ligand. CCCTC-binding factor (CTCF), promotor, enhancer, and promotor flank region were all shown to be involved in GLP-1R regulation. Furthermore, the GLP-1R variants are missense, coding sequence, synonymous, and intron (Ensembl 2022). GLP-1R was highly expressed in the pancreas with a median TPM score of 4.680 and the lowest expression in the esophagus-gastroesophageal Junction with a median TPM score of 0.01300. In the several organs GLP-1R with median TPM score more than 0.1 was expressed in heart - atrial appendage (1.748), nerve – tibial (0.9134), testis (0.7715), heart - left ventricle (0.5898), brain - caudate (basal ganglia) (0.5139), stomach (0.4197), brain – hypothalamus (0.3931), lung (0.3281), thyroid (0.3254), brain - nucleus accumbens (basal ganglia) (0.2772), minor salivary gland (0.2538), and brain - putamen (basal ganglia) (0.2425), skin - sun-exposed (Lower leg) (0.2187), skin - not sun-exposed (suprapubic) (0.1467), artery (0.1398) - tibial breast - mammary tissue (0.1396), brain – cortex (0.1293), respectively (Figure 4A).

The GTEx database contains a well-known database containing all gene expression profiles for human organs (Ardlie *et al.* 2015). GTEx provided the location and exon expression for GLP-1R (GTExPortal 2022). GLP-1R was particularly abundant in β-cells, to drive glucose-dependent insulin secretion and was present in α-cells, mediated the inhibition of glucagon secretion. GLP-1R is abundant in the vasculature of the cardiovascular system, mainly in arteries and arterioles where GLP-1 exerts vasodilatory effects (Richards *et al.* 2014). Alpha cell-specific GLP-1R knockout in mice has shown that the receptor does not affect beta-cell function, but it affects glucagon secretion in a glucose-dependent bidirectional

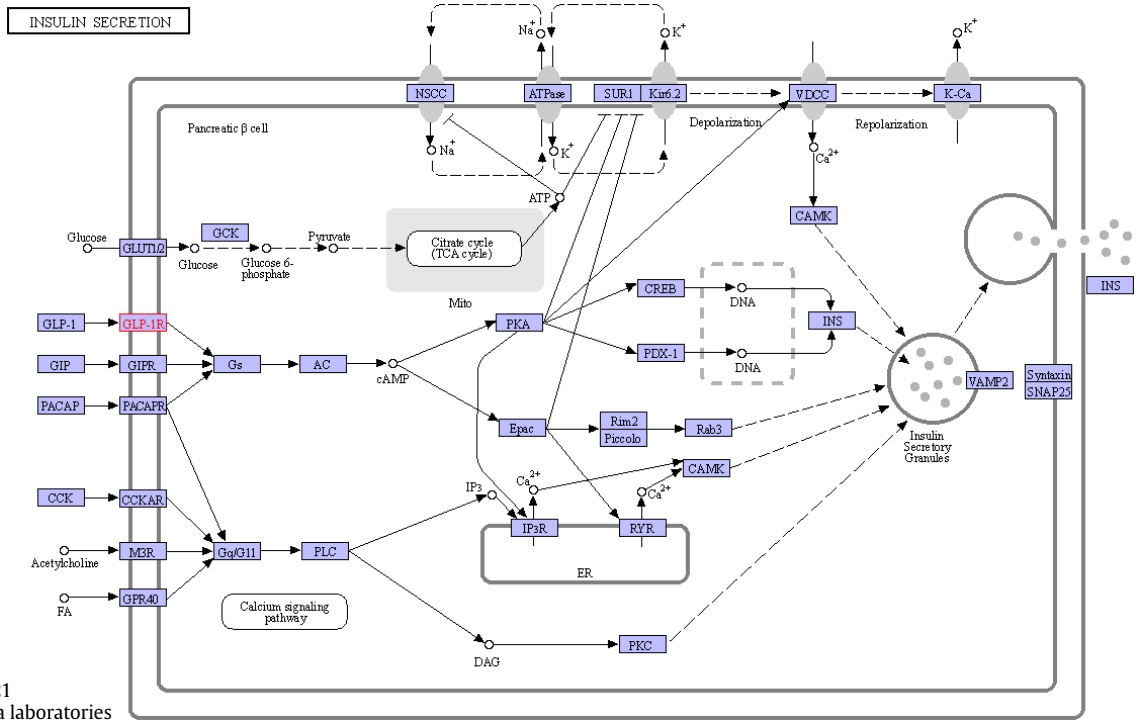

Figure 5. Insulin secretion pathways and function of GLP-1R by KEGG database

Table 1. 13 nsSNPs including their ID, allele change, protein accession number, its position, amino acid change and global MAF (Word)

SNP ID	Allele change	Protein accession	Position	Residue change
rs2295006	CGC => CAC	NP_002053.3	44	R (Arg) => H (His)
rs3765467	CGA => CAA	NP_002053.3	131	R (Arg) => Q (Gln)
rs6918287	GAA => GAG	NP_002053.3	133	E (Glu) => E (Glu)
rs6923761	GGC => AGC	NP_002053.3	168	G (Gly) => S (Ser)
rs10305492	GCC => ACC	NP_002053.3	316	A (Ala) => T (Thr)
rs1042044	TTA => TTC	NP_002053.3	260	L (Leu) => F (Phe)
rs2235868	AGG => CGG	NP_002053.3	176	R (Arg) =>R (Arg)
rs10305420	CCG => CTG	NP_002053.3	7	P (Pro) => L (Leu)
rs10305421	AGG => AAG	NP_002053.3	20	R (Arg) => K (Lys)
rs10305510	CGG => CAG	NP_002053.3	421	R (Arg) => Q (Gln)
rs140642887	GCC => ACC	NP_002053.3	239	A (Ala) => T (Thr)
rs146340667	GTC => ATC	NP_002053.3	194	V (Val) => I (Ile)
rs201672448	AGT => AAT	NP_002053.3	445	S (Ser) => N (Asn)

Table 2. The results for all 13 nsSNPs by five *in silico* tools

SNP ID	Amino acid change	SIFT		Polyphen2		PROVEAN		PhD-SNP
		Prediction	Tolerance index	Effect	Score	Score	Prediction (cutoff = -2.5)	Prediction
rs2295006	R44H	Tolerated	0.13	Benign	0.002	-0.614	Neutral	Disease
rs3765467	R131Q	Tolerated	0.35	Benign	0.019	0.358	Neutral	Neutral
rs6918287	E133E	N/A	N/A	N/A	N/A	0.000	Neutral	N/A
rs6923761	G168S	Tolerated	0.59	Benign	0.000	-0.665	Neutral	Disease
rs10305492	A316T	Tolerated	0.19	Benign	0.406	-2.593	Deleterious	Neutral
rs1042044	L260F	Tolerated	1.00	Benign	0.000	3.423	Neutral	Disease
rs2235868	R176R	N/A	N/A	N/A	N/A	0.000	Neutral	N/A
rs10305420	P7L	Tolerated	1.00	Benign	0.001	-1.217	Neutral	Neutral
rs10305421	R20K	Tolerated	0.29	Possibly damaging	0.643	-0.130	Neutral	Neutral
rs10305510	R421Q	Tolerated	0.28	Benign	0.007	-1.227	Neutral	Disease
rs140642887	A239T	Damaging	0.00	Possibly damaging	0.643	-3.685	Deleterious	Disease
rs146340667	V194I	Tolerated	0.60	Benign	0.020	-0.314	Neutral	Neutral
rs201672448	S445N	Tolerated	0.34	Possibly damaging	0.534	-0.498	Neutral	Neutral

Table 3. DDG values as predicted by I-Mutant 3.0

Amino acid	SVM2 prediction effect	DDG value (Kcal/mol)	DDG value (Kcal/mol)
A239T	Decrease	8	-0.76

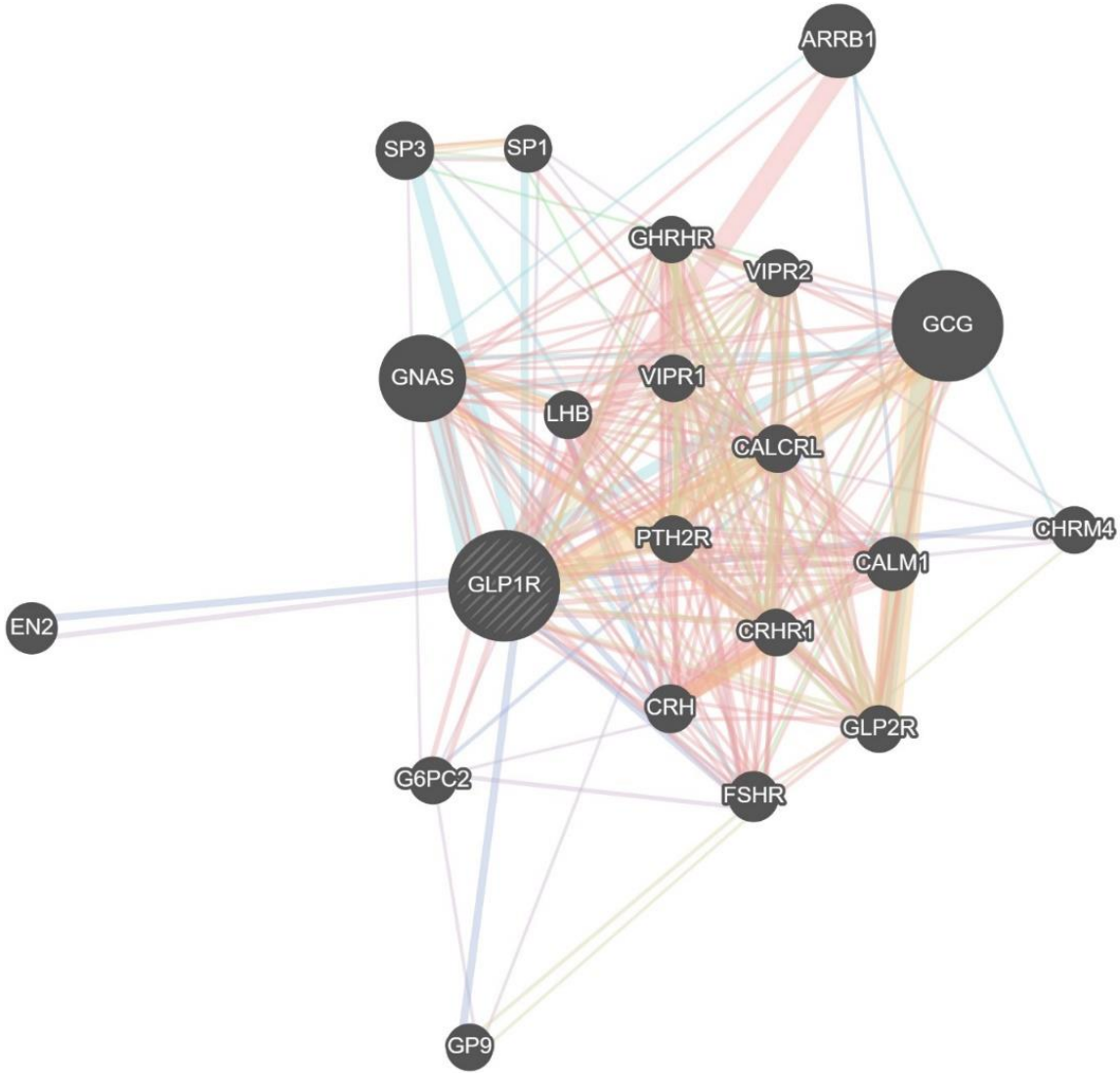

Figure 6. The GLP-1R gene interaction network predicted by GeneMANIA

way. It suggests that the receptor directly affects alpha cell function (Zhang *et al.* 2019) (Figure 4B).

A previous study reported that GLP-1R and glucagon receptor (GCGR) loss in signalling from intra-islet proglucagon-derived peptides decreased insulin production via reduced beta-cell cAMP synthesis, with input from alpha cells needed to maintain glucose tolerance under metabolic stress (Capozzi *et al.* 2019). In insulin secretion pathways, through the binding of GLP1 to GLP-1R, elevates cAMP levels and stimulates insulin secretion by the joint action of PKA and Epac2 enzymes, which stimulates the exocytotic process. PKA interacts with CREB and PDX1, activating insulin gene transcription and also has the additional effect of mobilizing intracellular Ca2+ through the activation of VDCC receptors (Figure 5). Several studies have been reported on associations between GLP-1R gene polymorphisms and many diseases, such as insulin secretion function (Weizheng *et al.* 2020), T2DM (Lin *et al.* 2015; Ma *et al.* 2018), obesity (Jensterle *et al.* 2015; Luis *et al.* 2014; Xu *et al.* 2021; Yau *et al.* 2018), bone mineral density (BMD) (Zhang *et al.* 2022),

coronary artery disease (Ma *et al.* 2018), and stress-related psychiatric disorders (Sheikha *et al.* 2010).

Four bioinformatics tools were used to investigate the effects of 13 missense SNPs nsSNPs on GLP-1R protein function and stability (Table 2). The missense SNPs were submitted to the SIFT software tool, which predicted that nsSNPs were damaging with a score ≤0.05 and tolerated with a score ≥0.05. The results of polyPhen2 indicated nsSNPs that were probably damaging, possibly damaging, and benign. Based on position-specific independent count score differences. PROVEAN tools predict deleterious variants below -2.5 and neutral variants above this threshold. rs10305421 and rs201672448 were damaged by polyphen2, rs10305492 was deleterious by PROVEAN, and PhD-SNP predicted rs2295006, rs6923761, rs1042044, and rs10305510 as diseased. Classification of nsSNPs as high-risk if four or more SNP prediction algorithms indicated deleterious or disease (Jenna and Stephen 2014). SIFT, PolyPhen-2, Provean, and PhD-SNP predicted that rs140642887 is deleterious, destabilizes, and alters the function of GLP-1R. nsSNPs positioned in highly conserved amino acid locations are often more deleterious than nsSNPs in non-converted sites, especially those involved in important biological processes (Miller and Kumar 2001). The rs140642887 variant was located in exon 7 of 13, the coding DNA sequence position was 715, and the amino acid alanine was altered to threonine in protein position 239.

According to the I-Mutant tool, nsSNPs rs140642887 was unstable and decreased GLP-1R protein stability (Table 3). Decreased protein stability increases protein degradation, misfolding, and aggregation (Singh *et al.* 2010). No study has been reported that demonstrates a link between the harmful nsSNPs rs140642887 and any disease. As a result, to complement this discovery, the confirmation of these nsSNPs in any disease is necessary.

Interactions between genes have become critical to predicting genes with distinct DNA sequence polymorphisms. Each has a unique set of wild-type and variant alleles and genotypes that primarily influence disease risk through interactions with genetic and environmental factors (Gilbert and Moore 2011; Warde *et al.* 2010). In addition to the frequency of path ending in a query node, the scores provided for each gene pair reflect the length and weighting of those pathways. GLP-1R coexpressed with engrailed homeobox 2 (EN2), cholinergic receptor muscarinic 4 (CHRM4), follicle-stimulating hormone receptor (FSHR), calcitonin receptors like receptor (CALCRL) and Sp1 transcription factor (SP1). GLP-1R has a strong pathway connection with SP1, Sp3 transcription factor (SP3), GNAS complex locus (GNAS) and glucagon (GCG). The most statistically significant (Q-value) assigned functions of GLP-1R are G protein-coupled receptor signalling pathway, coupled to cyclic nucleotide second messenger ($Q = 5.1 \times 10^{-15}$), hormone binding ($Q = 1.3 \times 10^{-11}$), peptide receptor activity ($Q = 3.7 \times 10^{-11}$), G protein-coupled receptor activity ($Q = 1.5 \times 10^{-10}$), and peptide binding ($Q = 3.7 \times 10^{-7}$) (Figure 6). Deleterious SNPs in the GLP-1R gene may impair the interaction and function of other genes in the network of gene-gene interactions. Physicochemical and molecular properties of the variant residues and the conservation of the structure are important for preserving and executing specific functions.

In conclusion, the conclusion of this study is GLP-1R strongly expressed in the pancreas and regulated glucose-dependent insulin secretion and mediated the inhibition of glucagon secretion. rs140642887 was deleterious, destabilising and altering the function of GLP-1R (A239T) by SIFT, PolyPhen2, Provean, and PhD.SNP. rs140642887 was unstable and reduced the stability of the GLP-1R protein. GLP-1R has a strong pathway connection with SP1, SP3, GNAS, and glucagon and also functions as a G protein-coupled receptor signalling pathway, coupled to cyclic nucleotide second messenger, hormone binding, peptide receptor activity, G protein-coupled receptor activity, and peptide binding.

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